UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: September 21, 2017

SUBJECT: **2, 4-D**. Review of 4-Week Inhalation Toxicity Study.

PC Code: 030001
Decision No.: 531960
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0057631
MRID No.: 50320801

DP Barcode: D441830
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: 94-75-7
40 CFR: 40 CFR §180.142

Ver.Apr. 2010

FROM: Linda Taylor, Ph.D.
Risk Assessment Branch VII
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THROUGH: Michael Metzger, Branch Chief
Risk Assessment Branch VII
Health Effects Division (7509P)
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TO: Christian Bongard, Risk Manager Reviewer
Risk Management and Implementation Branch III (RMIB III)
Pesticide Re-evaluation Division (7508P)
Office of Pesticide Programs

I. CONCLUSION: The 4-week inhalation toxicity study (MRID 50320801) on 2, 4-D has been reviewed, and a Data Evaluation Record (DER) has been prepared. The DER is appended.

II. ACTION REQUESTED: The Pesticide Re-Evaluation Division requested HED to review the 4-week inhalation toxicity study for 2, 4-D.

III. BACKGROUND: The registrant (The Dow Chemical Company) submitted a 4-week inhalation toxicity study (MRID 50320801), which was designed to evaluate the time-dependency of recovery from treatment-related, point-of-contact epithelial hyperplasia, focal

squamous metaplasia and hyperkeratosis, and mixed inflammatory cell influx observed in the larynx of male Sprague Dawley (Crl;CD(SD)) rats in the previous repeat dose inhalation study on 2,4-D (MRID 47398701; 2008).

IV. RESULTS/DISCUSSION: HED has reviewed the 4-week inhalation toxicity study on 2, 4-D and generated a DER (copy attached). The study is listed in the table below, along with a brief summary of the study results.

MRID Summary Table		
Study Type	**MRID (date)**	**Results/Classification**
870. 3465 Subchronic (28-day) inhalation toxicity (Sprague-Dawley Crl:SD rat) .	50320801 (2017) Acceptable/non-guideline 0, 0.05, 0.10, or 0.3 mg/L (males; nose only; 6 hours/day, 5 days/week for 4 weeks)	NOAEC = not identified. LOAEC = 0.05 mg/L, based on laryngeal lesions, including persistent incidence of squamous metaplasia of the mucosal epithelium at the base of the epiglottis and inflammation in the laryngeal mucosa at the base of the epiglottis, which were evident following Week 4 of recovery.

DATA EVALUATION RECORD

2, 4-DICHLOROPHENOXYACETIC ACID (2, 4-D)

Study Type: OCSPP No Guideline; OECD 412; 28-Day Inhalation Toxicity Study in the Rat

EPA Contract No. EP-W-16-018
Task Assignment No. 30-2-037 (MRID 50320801)

Prepared for
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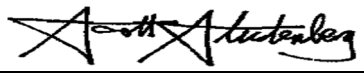
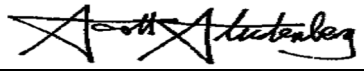
Primary Reviewer: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 07/19/2017
Secondary Reviewer: Michael E. Viana, Ph.D., DABT	Signature: Date:	 07/30/2017
Quality Assurance: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 07/31/2017
Project Manager: Michael E. Viana, Ph.D., DABT	Signature: Date:	 07/31/2017

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel.

EPA Reviewer: Linda Taylor, Ph.D.
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Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (28-Day) Inhalation Toxicity in Rats; OCSPP None; OECD 412.

PC CODE: 030001 **DP BARCODE:** D441830
TXR#: 0057631 **SUBMISSION No.:** None

TEST MATERIAL (PURITY): 2, 4-Dichlorophenoxyacetic acid (98.5% a.i.)

SYNONYMS: 2, 4-D; 2-(2, 4-Dichlorophenoxy)acetic acid

CITATION: Hotchkiss, J.A., Bell, M.P., Hutchinson, K.L., *et al.* (2017). 2, 4-Dichlorophenoxy acetic acid: 4-week subacute nose-only inhalation toxicity study in Crl:CD(SD) rats with 1-, 2-, and 4-week recovery groups. Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 161009, March 14, 2017. MRID 50320801. Unpublished.

SPONSOR: Industry Task Force II on 2, 4-D Research Data, c/o Mckenna Long & Aldridge LLP, 1900 K Street NW, Washington, DC.

EXECUTIVE SUMMARY: In a 28-day subchronic inhalation toxicity study (MRID 50320801), groups of 28 male Crl:CD(SD) rats/concentration were exposed to 2,4-dichlorophenoxyacetic acid (2,4-D; 98.5% a.i.; Batch # ENBK-143846-038) by nose only inhalation at concentrations of 0, 0.05, 0.1, or 0.3 mg/L (equivalent to 0, 50, 100, or 300 mg/m^3) for 6 h/day, 5 days/week for four weeks. The rats were exposed for a total of 20 days to evaluate the potential for exposure-dependent laryngeal lesions that were observed in a previous subchronic inhalation toxicity study in rats (MRID 47398701, 2008). The present study was designed specifically to evaluate the time-dependency of recovery from concentration-dependent, point-of-contact epithelial hyperplasia, focal squamous metaplasia and hyperkeratosis, and mixed inflammatory cell influx in the larynx of male Sprague Dawley CD® rats that were observed previously following repeated inhalation of a solid aerosol of 2,4-D. Post-exposure recovery groups of one, two, and four weeks were included to assess the persistence, or time-dependent recovery, of exposure-induced laryngeal lesions. Due to the specific purpose of the study, only in-life observations, body weight measurements, macroscopic evaluations, and detailed histopathologic examinations of the larynx only were performed.

No mortality, clinical signs of toxicity, adverse effects on body weight, or gross macroscopic findings were reported in any of the treatment groups.

Treatment-related microscopic changes to the laryngeal mucosa were reported in all 2, 4-D treatment groups along the anterior to posterior gradient. The base of the epiglottis (Level I) was the most sensitive site, with effects extending posteriorly towards the vocal processes of the arytenoid cartilage (Level II) in some rats. No treatment-related changes were reported in the posterior larynx at the cricoid cartilage (Level III).

At the end of the exposure phase, slight to moderate squamous metaplasia of the mucosal epithelium at the base of the epiglottis was observed in all rats at all dose levels. None of the control rats displayed this lesion, which was characterized by replacement of normal respiratory epithelium at the base of the epiglottis by keratinized stratified squamous epithelium up to 8-9 cell layers thick, depending on the severity. Squamous metaplasia extended posteriorly to the luminal epithelium at Level II. Concentration-dependent incidences of very slight or slight squamous metaplasia of the lateral surface of the arytenoid cartilage also were reported in all exposure groups (not observed in control) variably involving other portions of the luminal epithelium, including the epithelium overlying the U-shaped cartilages located dorsal to the ventral pouch. Very slight to slight hyperkeratosis was observed on the metaplastic squamous epithelium of the mucosa at Level I (all treated rats except 1 high dose; not in control), variably extending to Level II over the arytenoid cartilage (not observed in control). In addition, a dose-related increase in the incidence of very slight to slight hyperplasia of pre-existing squamous epithelium lining the medial aspect of the arytenoid cartilage was noted in the 0.1 and 0.3 mg/L treatment groups. Associated with these changes, slight to moderate, subacute or chronic inflammation was observed in the laryngeal mucosa at the base of the epiglottis (all treated rats and 4/10 controls), variably extending posteriorly to Level II involving the lamina propria of the arytenoid cartilage (not observed in control). The type, severity, and location of the laryngeal lesions observed in the present study were similar to findings in the previous four-week inhalation study (MRID 47398701).

Recovery Assessment. In the 0.3 mg/L recovery animals, the incidence (100%) and severity (slight to moderate) of squamous metaplasia of the mucosal epithelium at the base of the epiglottis following 1 week of recovery were the same as observed following the 4-week exposure period, and the incidence and severity were only slightly less following the 2- and 4-week recovery periods. Additionally, the incidence and severity of inflammation in the laryngeal mucosa at the base of the epiglottis were similar throughout the recovery period to that observed at the end of the four-week exposure period. Hyperkeratosis of the mucosal epithelium observed in the majority of 0.3 mg/L rats following the exposure period persisted at a reduced incidence and severity through Week 2 of recovery. Squamous metaplasia at the arytenoid epithelium at Level II was observed throughout the recovery period, although the incidence was less than that observed following the exposure period. Inflammation in the lamina propria of the arytenoid cartilage also were observed throughout the recovery period but the incidence and severity were decreased.

At 0.1 mg/mL, the incidence (100%) of squamous metaplasia of the mucosal epithelium at the base of the epiglottis following 1 week of recovery was the same as observed following the 4-week exposure period, and the severity was similar. The incidence was the same as that observed at 0.3 mg/mL following the 2-week recovery period and was still observed at both dose levels following the 4-week recovery period. Additionally, the incidence and severity of inflammation in the laryngeal mucosa at the base of the epiglottis were similar throughout the recovery period to that observed at the end of the four-week exposure period. Hyperkeratosis of the mucosal epithelium observed in all of the 0.01 mg/mL rats following the exposure period persisted through Week 2 of recovery. Squamous metaplasia at the arytenoid epithelium at Level II was observed in

2 rats following the 2-week recovery period.

At 0.05 mg/mL, squamous metaplasia of the mucosal epithelium at the base of the epiglottis, which was observed in all of the rats at the end of the exposure period, persisted throughout the recovery period (50%-83%), with 50% of the animals showing this lesion at the end of the 4-week recovery period. Inflammation in the laryngeal mucosa at the base of the epiglottis was observed in all of the rats at the end of the exposure period and throughout the recovery period, with 50% showing the lesion at the end of the four-week exposure period.

The LOAEC is 0.05 mg/L (equivalent to 50 mg/m^3), based on laryngeal lesions, including persistent incidence of squamous metaplasia of the mucosal epithelium at the base of the epiglottis and inflammation in the laryngeal mucosa at the base of the epiglottis, which were evident following Week 4 of recovery. The NOAEC was not identified.

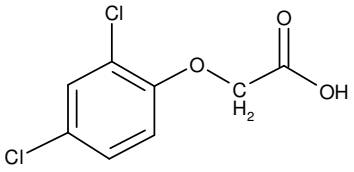
This study is classified **acceptable / nonguideline** and satisfies the stated purpose of evaluating the time-dependency of recovery from concentration-dependent, point-of-contact epithelial hyperplasia, focal squamous metaplasia and hyperkeratosis, and mixed inflammatory cell influx in the larynx of male Sprague Dawley rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided. It was stated that an exception to GLP compliance was that the GLP characterization of the test material was concurrent with the study, but that there was no negative impact to the study as the test material purity and ID were determined, and met expectations based on previous studies conducted with this test material.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: 2,4-Dichlorophenoxyacetic acid (2,4-D)

Description:	Not provided
Batch #:	ENBK-143846-038
Purity:	98.5% a.i.
Compound stability:	It was stated that the stability of the test material was nine years stored at room temperature.
CAS # of TGAI:	94-75-7
Structure:	

2. Vehicle: none

3. Test animals

Species:	Rat (male only)
Strain:	Sprague Dawley [Crl:CD(SD)]
Age/weight at study initiation:	Approximately 8 weeks / 208.0-270.3 g males
Source:	Charles River (Raleigh, NC)
Housing:	Two rats/cage in stainless steel cages with solid floors and corncob bedding
Diet:	LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, MO), *ad libitum* (except during exposure)
Water:	Tap water, *ad libitum* (except during exposure)
Environmental conditions:	**Temperature:** 20-26°C **Humidity:** 30-70% **Air changes:** Approximately 10-15/h **Photoperiod:** 12 h light / 12 h dark
Acclimation period:	Approximately two weeks

B. STUDY DESIGN

1. In life dates: Start: March 7, 2016 End: April 29, 2016

2. Animal assignment: Animals were stratified by body weight prior to the start of the study, and randomly assigned to treatment groups, as noted in Table 1, with a computer program.

TABLE 1: Study design [a]					
Test group	**Nominal concentration (mg/L)**	**Analytical concentration (mg/L)**	**MMAD [b] µm**	**GSD [c]**	**Rats [d]**
Control	0	0.0±0.0 [0.00][e]	-	-	28
Low	0.05	0.04±0.01 [0.02-0.08]	1.93±0.31	1.90±0.21	28
Mid	0.1	0.11±0.02 [0.05-0.18]	2.34±0.45	1.69±0.17	28
High	0.3	0.27±0.03 [0.19-0.47]	2.05±0.39	1.78±0.18	28

a Data were obtained from pages 23, 28 and 87 of MRID 50320801.
b MMAD = mass median aerodynamic diameter; mean±SD calculated by the reviewers.
c GSD = geometric standard deviation (σg); mean±SD calculated by the reviewers.
d Four-week terminal groups (n=10); additional recovery groups (n=6) terminated at 1-week, 2-week, and 4-week periods (Weeks 5, 6, and 8) after study initiation.
e [range of daily concentrations (mg/L)

3. Concentration selection rationale: The target exposure concentrations of 0.05, 0.10, and 0.30 mg 2, 4-dichlorophenoxyacetic acid (2, 4-D)/L air (6 h/day and 5 days/week for twenty exposure days) were selected to confirm and provide additional exposure-response results based on a previous four-week nose-only inhalation exposure study conducted with Sprague-Dawley rats (MRID 47398791, 2008[1]) with the same concentrations. The present study was conducted such that the anatomic location, character, and severity of laryngeal lesions resulting from repeated nose-only inhalation of aerosolized 2, 4-D in rats could be confirmed, and the time of recovery for these effects could be assessed. The high-exposure concentration (0.3 mg/L) was expected to induce slight to moderate squamous metaplasia in the ventral surface epithelium lining the anterior larynx at the base of the epiglottis (with minimal to moderate hyperkeratosis), minimal to moderate epithelial hyperplasia in the more distal arytenoid epithelium, and mixed inflammatory cell influx. The lower concentrations (0.1 and 0.05 mg/L) were expected to produce lesions of lesser to minimal severity.

4. Generation of the test atmosphere / chamber description: A diagram of the exposure generation system was provided on page 44, and is included as Appendix 1 at the end of this DER. Dow-modified, ADG flow-past, nose-only chambers (42-L, 30 cm diameter × 60 cm high) were used for the study. Room temperature, humidified, compressed air was supplied to the chamber. Airflow through the chamber was monitored with a manometer that measured the pressure drop across a calibrated orifice and maintained the flow at approximately 29-42 L/min, which was sufficient to provide normal oxygen concentrations with approximately 41-60 air changes/h. The manometers were calibrated with a gas meter prior to the start of the study.

The 2, 4-D exposure chambers were operated at a slightly negative pressure (relative to ambient atmospheric pressure) within a secondary, vented area. The control chamber was operated at a slightly positive pressure (relative to ambient atmospheric pressure) in a separate room to minimize the potential for 2, 4-D exposure. Chamber and exposure room temperatures were recorded from two thermocouples attached to an electronic thermometer, one located in or near the respective chambers. Relative humidity in each exposure chambers was monitored by an interior hygrometer. Dilution air for the chambers was passed through passive, stainless steel humidifiers filled with distilled water to humidify the dry, compressed air from the aerosol generator.

[1] Hoffman, G. M. (2008). A 28-day subchronic inhalation toxicity study of 2,4-dichlorophenoxyacetic acid in the rat *via* nose-only exposures. Huntingdon Life Sciences for the Industry Taskforce II on 2,4-D Research Data. MRID 47398701.

Based on the approximate airflow rate for the exposure chambers, the theoretical equilibrium time to 99% (T_{99}) of the target concentration was determined to be 4.6-6.7 min. The animals were connected to the chamber system after the T_{99} had elapsed, and were removed after 360 min of exposure/day.

A respirable (dust) aerosol of 2, 4-D was generated with a solid aerosol generator (Jet Mill Model 00 Jet-O-Mizer, Fluid Energy Aljet, Plumsteadville, Pennsylvania). The 2, 4-D was received milled, with a nominal particle size 1.03 µm (90% of particles were ≤ 2.95 µm), and was delivered continuously to the jet mill by using an auger-type bulk feeder. The jet mill de-agglomerated and aerosolized the test material, and a cyclone (5-µm cut off) was placed between the jet mill and the elutriation chamber to further reduce the particle size of the aerosol in the chamber. The 2, 4-D aerosol was drawn from the elutriation chamber and mixed with humidified dilution air within each exposure chamber. The ratio of aerosolized 2, 4-D aerosol and dilution air was adjusted to achieve the desired target concentration in each ADG, nose-only exposure chamber.

Test atmosphere concentration: The achieved analytical concentrations are presented in Table 1. The concentration of aerosol present in each chamber was determined at least three times during each six-hour exposure period for each exposure group. Each sample was collected by drawing chamber atmosphere from within the animal breathing zone at a set rate with a constant flow air sampling pump and collecting the aerosol particles on pre-weighed 47 mm glass-fiber filters. After each atmosphere sampling, the filter was re-weighed to determine the total weight of the particles, and the time-weighted average (TWA) exposure concentration was calculated from the gravimetric measurements for each chamber. Prior to test material exposure with animals, the distribution of the test material in the breathing zone of each chamber was checked from at least three sample points per breathing zone, and the normal sampling point (reference point) in each chamber. A nominal concentration for the exposure chambers was calculated from the amount of test material used in each generation apparatus and the total chamber airflow.

Particle size determination: Experimental particle size parameters are presented in Table 1. The aerodynamic particle size was determined at least twice per week for each exposure chamber by collecting samples from within the animal breathing zone at a set rate with a constant flow air sampling pump through a multi-stage cascade impactor. The MMAD and geometric standard deviation (GSD: σg) was determined for each sample.

5. Statistics: The following statistical analyses were conducted.

Parameter	Analysis
Body weight and body weight gain	Mean±SD; Bartlett's test for equality of variances; exploratory data analysis was conducted with a parametric ANOVA, if significant (alpha = 0.05) then Dunnett's test at alpha = 0.05 (experiment-wise error).
Exposure room temperature, and chamber concentration, temperature, relative humidity, and airflow	Mean±SD; statistical outliers (alpha = 0.02) identified by the sequential method of Grubbs (1969) were not excluded.

It was stated that because numerous measurements were compared statistically in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Interpretation of the data considered statistical analyses, as well as dose-

response relationships, the consistency of the results with other biological and pathological findings, and historical control values. The analyses were considered appropriate.

C. METHODS

1. Observations: Animals were observed for morbidity, mortality, and the availability of feed and water at least twice daily. Cage-side examinations were conducted at least once per day, approximately at the same time each day. Examinations were typically conducted with the animals in their cages, and the animals were not hand-held unless necessary. These examinations were meant to detect significant clinical abnormalities that were notable on limited observation, and to monitor the general health of the animals. Significant abnormalities that could have been observed included, but were not limited to: decreased/increased activity; repetitive behavior; vocalization; incoordination/limping; injury; neuromuscular function (convulsion, fasciculation, tremor, and twitches); altered respiration; pale skin and mucous membranes; severe eye injury; alterations in fecal consistency; and fecal/urinary quantity.

In addition, animals were observed for exposure-related effects approximately every hour during each daily 6-hour exposure period.

2. Body weight: All rats were weighed prior to exposure, a minimum of once during the first week of exposure, and then weekly for the remainder of the exposure and recovery periods. Cumulative body weight gains (relative to Day 1) were calculated for each interval.

3. Toxicokinetics: Individual blood samples for possible toxicokinetic analyses were collected from ten animals/group (40 total) immediately after exposure on a single day during the final week of exposure. Samples were taken by tail nick, and blood was collected in heparinized tubes. The blood samples were centrifuged, and plasma was harvested, transferred to glass vials, and stored frozen at −80°C. Evaluations for toxicokinetics were not reported.

4. Sacrifice and pathology: At the end of the exposure period, surviving rats were euthanized *via* exsanguination by severing the abdominal aorta under isoflurane/O_2 anesthesia. All animals that were euthanized moribund, and those euthanized on schedule, were subjected to a gross pathological examination. The necropsy included an examination of the external tissues and all orifices. The head was removed, the cranial cavity opened, and the brain, pituitary, and adjacent cervical tissues were examined. The skin was reflected from the carcass, the thoracic and abdominal cavities were opened, and the viscera examined. All visceral tissues were dissected from the carcass, re-examined, and the CHECKED (X) tissues were collected and preserved. Organ weight data were not collected.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	X	Brain*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve (tibial)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*+		**GLANDULAR**
X	Ileum*			X	Adrenal gland*+
X	Cecum*		**UROGENITAL**	X	Lacrimal gland
X	Colon*	X	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
X	Liver*+	X	Testes*+		**OTHER**
X	Oral tissues	X	Epididymides*+	X	Bone (including joint)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin (and subcutis)
	RESPIRATORY	X	Coagulation gland	X	All gross lesions and masses*
X	Trachea*			X	Harderian gland
X	Lung*			X	Mediastinal tissues
X	Nasal tissue*			X	Mesenteric tissues
X	Pharynx*			X	Auditory sebaceous glands
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465
\+ Organ weights required

The nasal cavity was flushed through the nasopharyngeal duct, and the lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin with a hand-held syringe and blunt needle. Representative samples of tissues were collected and preserved in neutral, phosphate-buffered 10% formalin. Transponders (coded with unique alphanumeric ID numbers) were removed and placed in jars with the tissues.

Microscopic evaluation was restricted to the larynx. The larynx was cut transversely at three anatomic locations, and sections for microscopic examination were taken at each location [i.e., seromucinous glands at the base of the epiglottis (Level I), the arytenoid cartilage and ventral pouch (Level II), and the cricoid cartilage (Level III). The sets of tissue blocks were processed for light microscopy and detailed histopathologic analysis. A diagram of the anatomic sites that were examined in each larynx is included as Appendix 2 at the end of this DER. Hematoxylin and eosin stained sections from each level were examined microscopically, and selected histopathologic findings were graded to reflect the severity of specific lesions to evaluate: 1) the effects of specific lesions on larynx function, 2) exacerbation of naturally-occurring lesions due to exposure to the test material, and 3) dose-response relationships for treatment-related effects. The severity grade scale that was used to evaluate treatment-related microscopic changes in each larynx (see Appendix 2 at the end of DER). The categorical rankings of lesion severity were assigned numeric values (no change=0, very slight=1, slight=2, moderate=3, and severe=4). In addition, a lesion severity index (LSI) score (mean±SE) for each identified lesion was calculated for the treatment groups at each assessment period.

II. RESULTS

A. OBSERVATIONS

1. Mortality: No incidences of treatment-related mortality occurred in the present study. One accidental death due to suffocation (animal #676, 0.05 mg/L group) occurred during exposure on Day 21, and another 0.05 mg/L animal (#659) was humanely euthanized after accidental injury to the neck on Day 24. All other rats survived to scheduled euthanasia.

2. Clinical signs of toxicity: No clinical signs of toxicity were observed in any treatment groups.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight and body weight gain data are presented in Table 2. There were no treatment-related differences in body weights of any treatment groups compared to their respective controls during the exposure or recovery periods. Cumulative body weight gain (BWG) was decreased ($p<0.05$) by 10% for the Day 1-19 period for the 0.1 mg/L animals. A similar decrease (not significant, NS) of 10% was noted for the 0.3 mg/L animals during this period. For the cumulative Day 1-25 period (end of the four-week exposure phase), BWGs were decreased 11% and 12%, respectively, for the 0.1 and 0.3 mg/L animals compared to control. By Day 32 (end of the first week of recovery), cumulative BWGs in the 0.1 and 0.3 mg/L dose groups were similar to control, with no further BWG effects noted throughout the rest of the recovery period (Day 53).

TABLE 2. Mean (±SD) body weights and body weight gains (g) in male rats treated with 2, 4-D by nose-only inhalation for up to four weeks. [a]

Day	Concentration (mg/L)			
	0	**0.05**	**0.1**	**0.3**
1	243.1±13.1	243.4±12.9	246.4±12.9	242.9±15.7
5	262.3±16.1	265.3±13.8	264.0±16.1	261.6±18.2
12	301.0±19.6	305.1±17.6	299.2±20.7	298.3±21.7
19	332.7±23.3	335.8±20.3	327.0±24.4	323.6±24.9
25	351.5±26.8	355.5±25.7	342.8±27.0	338.4±28.8
1 [b]	242.2±13.4	244.3±12.7	244.9±12.8	242.4±16.9
32	373.5±27.3	387.0±29.4	365.1±29.2	367.2±33.7
53	435.4±39.4	462.8±46.7	439.1±35.4	445.0±54.1
BWG 1-12	57.9±10.7	61.8±8.8	52.8±11.6	55.4±8.4
BWG 1-19	89.6±15.0	92.4±12.2	80.6±16.1* (↓10)	80.7±11.9 (↓10)
BWG 1-25	108.4±19.0	111.6±18.0	96.4±18.9* (↓11)	95.5±16.9* (↓12)
BWG 1-32	131.3±19.5	142.7±22.4	120.3±22.7	124.8±20.0
BWG 1-53	201.0±24.6	224.0±37.9	197.0±23.3	200.4±34.9

a Data were obtained from Table 4 on pages 55-56; n=28 (Days 1-25, except for Day 25, 0.05 mg/L, n=26). Percent differences from controls (calculated by the reviewers) are presented in parentheses.
b Control values for recovery animals, n=18 (Days 1 and 32) and n=6 for Day 53.
* Significantly different from control; $p<0.05$.

C. TOXICOKINETICS: Toxicokinetic evaluations were not reported.

D. SACRIFICE AND PATHOLOGY

1. Gross pathology: There were no treatment-related macroscopic observations noted for any animals in the 2, 4-D exposure or recovery groups. All macroscopic observations were considered to be spontaneous alterations or lesions associated with inadvertent accidents and unrelated to 2, 4-D exposure.

Two animals were removed from the study for reasons unassociated with 2, 4-D exposure. One rat (#676) in the 0.05 mg/L group was found dead in the nose-cone on Day 21. There

were no gross macroscopic observations noted in this animal, and the cause of death was attributed to accidental suffocation due to the nose-cone. Another rat (#659) in the 0.05 mg/L group was euthanized on Day 24 due to accidental trauma to the neck. Post-mortem macroscopic observations for this animal consisted of hemothorax, hemorrhage of the skin and subcutis in the neck region, and gas-filled stomach.

2. **Microscopic pathology:** Microscopic evaluations in the present study were restricted to the larynx. The treatment-related microscopic observations of the larynx at the end of exposure (Week 4) are shown in Table 3 (Level I) and Table 4 (Level II). The lesion severity index (LSI) score for each identified lesion is included as Appendix 3 at the end of this DER.

No treatment-related changes were observed in Level III (posterior larynx at the cricoid cartilage) in any treatment group. Concentration-dependent microscopic changes to the laryngeal mucosa were observed in all 2, 4-D treatment groups along the anterior to posterior gradient. The base of the epiglottis (Level I) was the most affected site, with effects extending posteriorly towards the vocal processes of the arytenoid cartilage (Level II) in some rats.

Slight to moderate squamous metaplasia of the mucosal epithelium at the base of the epiglottis (referred to as ventral epithelium in the previous inhalation study) was observed in all rats at each dose level following the 4-week exposure period (Table 3). None of the controls displayed this lesion. Depending on the severity, the lesions were characterized by replacement of the normal respiratory epithelium (a mixture of ciliated cells and non-ciliated, round to cuboidal cells 2-3 cell layers thick) at the base of the epiglottis by keratinized, stratified squamous epithelium up to 8-9 cell layers thick. Concentration-dependent incidences of very slight to slight squamous metaplasia of the lateral surface of the arytenoid cartilage (Level II section of larynx) also were observed in all exposure groups, variably involving other portions of the luminal epithelium, including the epithelium overlying the U-shaped cartilages located dorsal to the ventral pouch (Table 4). None of the controls displayed this lesion. Very slight to slight hyperkeratosis was observed on the metaplastic squamous epithelium of the mucosa at Level I, extending to Level II over the arytenoid cartilage. None of the controls displayed hyperkeratosis at either level. A concentration-dependent increase in the incidence of very slight to slight hyperplasia of pre-existing squamous epithelium lining the medial aspect of the arytenoid cartilage was observed in the 0.1 and 0.3 mg/L treatment groups compared to the control. The hyperplasia was characterized by a very slight to slight increase in the overall thickness of the squamous epithelial cell layers lining the medial surface of the arytenoid cartilage. Slight to moderate inflammation in the laryngeal mucosa at the base of the epiglottis (Level I) was observed in all rats (8/8 or 10/10) at each dose level compared to the control incidence (very slight in 4/10 controls), which extended posteriorly to Level II involving the lamina propria of the arytenoid cartilage (not observed in control). The inflammatory response was characterized by the presence of a mixed inflammatory cell infiltrate composed of mononuclear cells, with small numbers of neutrophils and occasional eosinophils within the lamina propria of the affected sites.

Table 3. Incidence of Microscopic Changes (Level I) in the Larynx Following Exposure				
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**
# larynx examined	10	8	10	10
Metaplasia; squamous; epithelium; mucosa				
Slight	0	6	3	3
Moderate	0	2	7	7
Total incidence	0	8	10	10
Hyperkeratosis; epithelium; mucosa				
Very slight	0	7	7	6
Slight	0	1	3	3
Total incidence	0	8	10	9
Inflammation; mucosa				
Very slight	4	4	0	0
Slight	0	4	10	9
Moderate	0	0	0	1
Total incidence	4	8	10	10

a Data obtained from page 34 (Text Table 6).

Table 4. Incidence of Microscopic Changes (Level II) in the Larynx Following Exposure				
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**
# larynx examined	10	8	10	10
Metaplasia; squamous; epithelium; arytenoid				
Very slight	0	1	2	0
Slight	0	1	2	6
Total incidence	0	2	4	6
Hyperkeratosis; epithelium; arytenoid				
Very slight	0	1	3	4
Hyperplasia; squamous; epithelium; arytenoid				
Very slight	1	0	0	2
Slight	0	0	3	4
Total incidence	1	0	3	6
Inflammation; lamina propria				
Very slight	0	3	4	1
Slight	0	0	0	5
Total incidence	0	3	4	6

a Data obtained from page 34 (Text Table 6).

The type, severity, and location of the laryngeal lesions observed in the present study were similar to effects observed in the previous four-week inhalation study (see Table 5 below).

Table 5. Study Comparison of Microscopic Findings in Larynx - 4-Week Exposure Period								
Study	**MRID 47398701**				**MRID 50320801**			
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**	**0.00**	**0.05**	**0.10**	**0.03**
# larynx examined	10	10	10	10	10	8	10	10
Epithelium Squamous/Squamoid Metaplasia								
Minimal	0	0	0	0	-	-	-	-
Very slight	-	-	-	-	0	0	0	0
Slight	0	10	10	8	0	6	3	3
Moderate	0	0	0	2	0	2	7	7
Total incidence	0	10	10	10	0	8	10	10
Epithelium Hyperkeratosis								
Minimal	0	8	10	7	-	-	-	-
Very slight	-	-	-	-	0	7	7	6
Slight	0	0	0	2	0	1	3	3
Moderate	0	0	0	1	-	-	-	-
Total incidence	0	8	10	10	0	8	10	9
Mixed Inflammatory Cells/ Inflammation								
Minimal	5	1	1	1	-	-	-	-
Very slight	-	-	-	-	4	4	0	0
Slight	4	9	4	4	0	4	10	9
Moderate	0	0	5	5	0	0	0	1
Total incidence	9	10	10	10	4	8	10	10

- Severity ranking term not used

Partial recovery of the treatment-related lesions observed in larynxes was observed after one week of recovery, with further resolution of the lesions by Weeks 2 and 4 of recovery (Level I, Table 6; Level II, Table 7).

Table 6. Incidence of Microscopic Changes (Level I) in the Larynx Following Recovery												
	1-week recovery				**2-week recovery**				**4-week recovery**			
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**	**0.00**	**0.05**	**0.10**	**0.03**	**0.00**	**0.05**	**0.10**	**0.03**
# larynx examined	6	6	6	6	6	6	6	6	6	6	6	6
Metaplasia; squamous; epithelium; mucosa												
Very slight	0	2	2	0	0	3	3	0	0	3	2	4
Slight	0	1	3	5	0	2	2	5	0	0	0	1
Moderate	0	0	1	1	0	0	0	0	0	0	0	0
Total incidence	0	3	6	6	0	5	5	5	0	3	2	5
Epithelial alteration; mucosa; focal												
Very slight	0	2	0	0	0	1	0	1	0	0	1	1
Slight	0	1	0	0	0	0	0	0	0	0	0	0
Total incidence	0	3	0	0	0	1	0	1	0	0	1	1
Hyperkeratosis; epithelium; mucosa												
Very slight	0	0	1	3	0	0	0	2	0	0	0	0
Slight	0	0	0	0	0	0	0	0	0	0	0	0
Total incidence	0	0	1	3	0	0	0	2	0	0	0	0
Inflammation; mucosa												
Very slight	2	3	4	3	2	2	5	2	2	3	6	3
Slight	0	1	2	3	0	1	1	4	0	0	0	2
Moderate	0	0	0	0	0	0	0	0	0	0	0	0
Total incidence	2	4	6	6	2	3	6	6	2	3	6	5

From page 34 (Text Table 6).

Table 7. Incidence of Microscopic Changes (Level II) in the Larynx Following Recovery												
	1-week recovery				2-week recovery				4-week recovery			
2,4-D (mg/L)	**0.00**	**0.05**	**0.10**	**0.03**	**0.00**	**0.05**	**0.10**	**0.03**	**0.00**	**0.05**	**0.10**	**0.03**
# larynx examined	6	6	6	6	6	6	6	6	6	6	6	6
Metaplasia; squamous; epithelium; arytenoid												
Very slight	0	0	2	1	0	1	0	3	0	0	0	1
Slight	0	0	0	1	0	0	0	1	0	0	0	0
Total incidence	0	0	2	2	0	1	0	4	0	0	0	1
Epithelial alteration; arytenoid; focal												
Very slight	0	0	0	1	0	0	0	1	0	0	0	0
Total incidence	0	0	0	1	0	0	0	1	0	0	0	0
Hyperkeratosis; epithelium; arytenoid												
Very slight	0	0	0	1	0	1	0	0	0	0	0	0
Total incidence	0	0	0	1	0	1	0	0	0	0	0	0
Hyperplasia; squamous; epithelium; arytenoid												
Very slight	0	0	0	0	0	1	0	1	0	0	0	0
Total incidence	0	0	0	0	0	1	0	1	0	0	0	0
Inflammation; lamina propria; arytenoid												
Very slight	0	0	0	2	0	1	0	1	0	0	0	1
Slight	0	0	0	0	0	0	0	0	0	0	0	0
Total incidence	0	0	0	2	0	1	0	1	0	0	0	1

From page 34 (Text Table 6).

Treatment-related squamous metaplasia of the mucosal epithelium at the base of the epiglottis demonstrated progressive resolution over the 1-, 2-, and four-week recovery periods, although it was still evident at all dose levels following 4 weeks without exposure. Lesion severities were reduced in all treatment groups after one week of recovery. In the 0.05 mg/L treatment group, 50% of the animals had very slight squamous metaplasia following 4 weeks without exposure compared to 100% with slight to moderate squamous metaplasia at the end of treatment. In the 0.1 mg/L and 0.3 mg/L treatment groups, the severity of lesions also was reduced with 83% of the animals with slight or very slight squamous metaplasia per group following 2 weeks of recovery compared to 100% per group with slight to moderate squamous metaplasia at the end of the exposure period. At Week 4 of recovery, 50% or 33% of the animals in the 0.05 or 0.1 mg/L treatment groups, respectively, had very slight squamous metaplasia remaining. For the 0.3 mg/L animals, 83% had very slight or slight squamous metaplasia remaining at Week 4 of recovery.

The incidence and severity of squamous metaplasia of the arytenoid epithelium (lateral aspect; Table 7) resolved more rapidly, and to a greater extent, during the four-week recovery phase compared to the lesions present in the Level I mucosa. Squamous metaplasia of the arytenoid epithelium present at the end of the four-week exposure in 0.05 mg/L animals was resolved in all rats examined at Weeks 1 and 4 of recovery (lesion in 1 of 6 rats at Week 2 was very slight). In rats exposed to 0.1 mg/L, squamous metaplasia of the arytenoid epithelium was resolved by Week 2 of recovery. In rats exposed to 0.3 mg/L, slight squamous metaplasia observed in 6/10 rats at the end of the four-week exposure period was decreased in incidence at Week 1 (2/6), Week 2 (4/6), and Week 4 (1/6) and severity at Week 4 post exposure. This continued presence of squamous metaplasia at the arytenoid epithelium at Week 4 of recovery

in the 0.3 mg/L group, in addition to squamous metaplasia of the anterior mucosal epithelium (very slight in 4/6 animals and slight in 1/6 animals) is considered an adverse change.

Isolated incidences of very slight or slight epithelial alterations, characterized by flattened epithelium and lack of ciliated cells, were also observed in the mucosal epithelium (Level I) and arytenoid epithelium (Level II) during the four-week recovery period. Hyperkeratosis was observed in Levels I and II of all treatment groups at the end of exposure, and was fully resolved after Week 4 of recovery. Hyperplasia of the squamous epithelium of the arytenoid (Level II) in rats exposed to 0.1 and 0.3 mg/L for four weeks was resolved at Week 1 of recovery. Very slight hyperplasia of the squamous epithelium of the arytenoid (Level II) was noted in isolated animals (1 of 6) in the 0.05 and 0.3 mg/L groups at the end of Week 2 of recovery.

Treatment-related, very slight to slight (one high dose moderate), inflammation in the laryngeal mucosa at the base of the epiglottis (in all treated rats; 4/10 controls) and lamina propria of the arytenoid cartilage (0/6 controls; 3/6, 4/6, 6/6 with increasing dose) were observed in all treated groups at the end of the four-week exposure period. Inflammation was reduced in severity in all treated groups at Levels I and II at the end of Week 1 of recovery, and fully resolved in the 0.05 mg/L group at Level II. Very slight or slight inflammation persisted in the 0.1 mg/L (6/6; Level I) and 0.3 mg/L (5/6 and 1/6; Levels I and II, respectively) recovery groups at the end of the four-week recovery period. The inflammatory response was characterized by a minimal presence of mononuclear cells mixed with an occasional neutrophil in the mucosa at the base of the epiglottis.

TABLE 8. Incidence of microscopic changes in the larynx of rats treated with 2,4-D by nose-only inhalation for four weeks and following 1, 2, and 4 weeks without exposure. [a]

		End of Exposure				1-Week recovery				2-Week recovery				4-Week recovery			
	Exposure level (mg/L)	0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3
	Larynx: Number examined	10	8	10	10	6	6	6	6	6	6	6	6	6	6	6	6
Level I	Metaplasia; squamous; epithelium; mucosa																
	very slight	0	0	0	0	0	**2**	**2**	0	0	**3**	**3**	0	0	**3**	**2**	**4**
	slight	0	**6**	**3**	**3**	0	**1**	**3**	**5**	0	**2**	**2**	**5**	0	0	0	**1**
	moderate	0	**2**	**7**	**7**	0	0	**1**	**1**	0	0	0	0	0	0	0	0
	Epithelial alteration; mucosa; focal																
	very slight	0	0	0	0	0	**2**	0	0	0	**1**	0	**1**	0	0	**1**	**1**
	slight	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
	Hyperkeratosis; epithelium; mucosa																
	very slight	0	**7**	**7**	**6**	0	0	**1**	**3**	0	0	0	**2**	0	0	0	0
	slight	0	**1**	**3**	**3**	0	0	0	0	0	0	0	0	0	0	0	0
	Inflammation; subacute to chronic; mucosa																
	very slight	4	4	0	0	2	3	**4**	**3**	2	2	**5**	**2**	2	3	**6**	**3**
	slight	0	**4**	**10**	**9**	0	**1**	**2**	**3**	0	**1**	**1**	**4**	0	0	0	**2**
	moderate	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
Level II	Metaplasia; squamous; epithelium; arytenoid																
	very slight	0	**1**	**2**	0	0	0	**2**	**1**	0	**1**	0	**3**	0	0	0	**1**
	slight	0	**1**	**2**	**6**	0	0	0	**1**	0	0	0	**1**	0	0	0	0
	Epithelial alteration; arytenoid; multifocal																
	very slight	0	0	0	0	0	0	0	**1**	0	0	0	**1**	0	0	0	0
	Hyperkeratosis; epithelium; arytenoid																
	very slight	0	**1**	**3**	**6**	0	0	0	**1**	0	**1**	0	0	0	0	0	0
	Hyperplasia; squamous; epithelium; arytenoid																
	very slight	**1**	0	**3**	**2**	0	0	0	0	0	**1**	0	**1**	0	0	0	0
	slight	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	0	0
	Inflammation; subacute to chronic; lamina propria; arytenoid																
	very slight	0	**3**	**4**	**1**	0	0	0	**2**	0	**1**	0	**1**	0	0	0	**1**
	slight	0	0	0	**5**	0	0	0	0	0	0	0	0	0	0	0	0

a Text Table 6 from page 34 of the study report.
Bolded values indicate effects judged to be related to treatment (according to the study authors).

III. DISCUSSION AND CONCLUSIONS

A. <u>INVESTIGATORS CONCLUSIONS</u>: This study was conducted to confirm the exposure-response profile of 2, 4-D induced laryngeal lesions previously reported by Hoffman (2008) and to assess the concentration and time-dependency of lesion resolution (recovery) in exposure groups where recovery was not examined in the earlier study. Under the conditions of this study, repeated inhalation of a respirable solid (dust) aerosol of 2,4-D for 4 weeks resulted in concentration-dependent, point-of-contact, morphologic and inflammatory alterations to the anterior larynx of male Crl:CD(SD) rats that were similar in morphology, location, and severity to those reported by Hoffman (2008). The lesions were interpreted to be largely adaptive in nature and were markedly reduced in incidence and severity after 1 week of recovery, with continued lesion resolution over the full four-week recovery period. At the end of the four-week recovery period, exposure-related changes noted in the 0.1 mg/L recovery group were very slight focal squamous metaplasia and very slight subacute to chronic inflammation restricted to the mucosal epithelium at the base of the epiglottis, which were interpreted to be non-adverse due to the minimal severity and for negligible potential

laryngeal dysfunction. Therefore, based on the concentration-dependent cumulative lesion severity index, regional distribution of the initial lesions, and the magnitude and time-dependency of lesion resolution, 0.1 mg/L was determined to be the NOAEC for an inhaled 2, 4-D dust aerosol after exposure for four weeks.

B. REVIEWER COMMENTS: No mortality, clinical signs of toxicity, or body weight effects were observed in any treatment groups. Evaluations for toxicokinetics were not reported. No gross macroscopic findings were observed.

Concentration-dependent microscopic changes to the laryngeal mucosa were observed in all 2, 4-D treatment groups along the anterior to posterior gradient. The base of the epiglottis (Level I) was the most affected site for treatment-related changes, with effects extending posteriorly towards the vocal processes of the arytenoid cartilage (Level II) in some rats. These microscopic lesions were not observed in the controls except for very slight inflammation in Level I musosa. No treatment-related changes were observed in the posterior larynx at the cricoid cartilage (Level III) in any treatment group.

There was a concentration dependent increase in the severity of squamous metaplasia of the mucosal epithelium at the base of the epiglottis (in all rats at each dose level but not in controls), which extended posteriorly to the luminal epithelium at Level II. A concentration-dependent increase in the incidence of very slight to slight squamous metaplasia of the lateral surface of the arytenoid cartilage also were observed (all dose levels) involving other portions of the luminal epithelium, including the epithelium overlying the U-shaped cartilages located dorsal to the ventral pouch (not in controls). Very slight to slight hyperkeratosis was observed on the metaplastic squamous epithelium of the mucosa at Level I, extending to Level II over the arytenoid cartilage. In addition, there was a concentration-dependent increase in the incidence of very slight to slight hyperplasia of pre-existing squamous epithelium lining the medial aspect of the arytenoid cartilage in the 0.1 mg/L (50%) and 0.3 mg/L (100%) treatment groups. Slight to moderate inflammation was observed in all treated rats in the laryngeal mucosa at the base of the epiglottis (Level I), which extended posteriorly to Level II (very slight to slight) involving the lamina propria of the arytenoid cartilage. The type, severity, and location of the laryngeal lesions observed in the present study were similar to effects observed in a previous four-week inhalation study on 2, 4-D (MRID 47398701).

Partial recovery of the treatment-related lesions observed in larynxes of all rats treated with 2, 4-D was observed after one week of recovery, with further resolution of the lesions by Weeks 2 and 4 of recovery. Treatment-related squamous metaplasia of the mucosal epithelium at the base of the epiglottis demonstrated progressive resolution throughout the recovery periods. Lesion severities were reduced in all treatment groups after one week of recovery, with continued recovery to Week 4. At Week 4 of recovery, 3/6 and 2/6 animals in the 0.05 or 0.1 mg/L treatment groups, respectively, had focal regions of very slight squamous metaplasia remaining. For the 0.3 mg/L animals, 5/6 had focal regions of very slight (4/6) or slight (1/6) squamous metaplasia remaining at Week 4.

The incidence and severity of squamous metaplasia of the arytenoid epithelium (lateral aspect; Level II) resolved more rapidly and to a greater extent during the four-week recovery phase, compared to Level I. Squamous metaplasia of the arytenoid epithelium present at the end of the four-week exposure in 0.05 mg/L animals was resolved in all rats (6/6) examined at Week

1 of recovery, and no evidence of squamous metaplasia was observed in the four-week recovery group. In rats exposed to 0.1 mg/L, squamous metaplasia of the arytenoid epithelium was resolved by Week 2 of recovery. In rats exposed to 0.3 mg/L, slight squamous metaplasia observed in 6/10 rats at the end of the four-week exposure period was decreased in incidence and severity in all recovery groups, with very slight focal squamous metaplasia in 1/6 rats at Week 4. This continued presence of squamous metaplasia at the arytenoid epithelium at Week 4 of recovery in the 0.3 mg/L group, in addition to squamous metaplasia of the anterior mucosal epithelium (very slight in 4/6 animals and slight in 1/6 animals), is considered an adverse change.

Hyperkeratosis observed in Levels I and II of all treatment groups at the end of exposure was fully resolved in the 0.05 mg/L animals after one week of recovery (except for 1 of 6 animals with focal, very slight hyperkeratosis in Level II at Week 2 of recovery). Hyperkeratosis in rats exposed to 0.1 or 0.3 mg/L was reduced in incidence and severity at Week 1 of recovery, and fully resolved after Weeks 2 or 4 of recovery, respectively.

Hyperplasia of the squamous epithelium of the arytenoid in rats exposed to 0.1 and 0.3 mg/L for four weeks was resolved at Week 1 of recovery. Very slight hyperplasia of the squamous epithelium of the arytenoid was noted in isolated animals (1 of 6) in the 0.05 and 0.3 mg/L groups at the end of Week 2 of recovery.

Treatment-related, very slight to moderate, inflammation in the laryngeal mucosa at the base of the epiglottis (all treated rats; 4/10 controls), and lamina propria of the arytenoid cartilage (only in treated), were observed at the end of the four-week exposure period. Inflammation was reduced in severity in all treated groups at both levels at the end of Week 1 of recovery, and fully resolved in the 0.05 mg/L group at Level II. The incidence of inflammation was still greater than in the control (33%) by Week 4 of recovery in the 0.05 mg/L group (50%) at Level I. Very slight or slight inflammation persisted in the 0.1 mg/L and 0.3 mg/L recovery groups at the end of the four-week recovery period, but was decreased in incidence and severity relative to the end of exposure.

During the Agency's review of the protocol for the current inhalation study, it was strongly recommended that a dose lower than 0.05 mg/L be incorporated in case recovery was not demonstrated at this dose level. It was concluded that the slight laryngeal metaplasia observed in the previous study at all dose levels may be assessed as non-adverse **if** it were found to be reversible in the new study. However, if recovery was not demonstrated at 0.05 mg/L, HED clearly emphasized that the findings observed in the original inhalation study at this concentration and above would be considered adverse.

The Agency's assessment of the previous inhalation study on 2, 4-D concluded that (1) Treatment-related increases of the incidence of more diffuse, moderate to severe squamous metaplasia at various levels of the larynx should be considered adverse. (2) The occurrence of a focal, squamous hyperplasia within an area of laryngeal squamous metaplasia is regarded to be an adverse finding. (3) In the larynx, squamous metaplasia and keratinization may lead to functional disorders, such as a reduced clearance and decreased tendency to cough. (4) Inflammatory responses due to irritant effects are regarded to be adverse findings.

Using these criteria, the findings at the end of exposure at the low dose in the current study

are considered adverse. At the low dose, there is more recovery than at the higher doses by 4 weeks' post-exposure: 8/8 metaplasia (2/8 moderate) to 3/6 metaplasia (0/6 moderate), 8/8 hyperkeratosis to 0/6 hyperkeratosis, and 8/8 inflammation (4/8 slight) to 3/6 inflammation (3/6 very slight vs. 2/6 very slight background inflammation in controls). However, 50% of the rats at 0.05 mg/L showed squamous metaplasia and 50% showed mucosal inflammation following 4 weeks of no exposure. Squamous metaplasia was not observed in the control group at any time point. Inflammation incidence was 40% in control vs 100% at all dose levels following the exposure period and 33% throughout recovery in controls vs 50%-67% at 0.05 mg/L. If assessed in isolation, it is possible to consider the low dose effects at the end of 4-week recovery period to be non-adverse. However, the ability of the test species to recover from damage (i.e., reversibility) is an important factor in an adversity decision. The results in the current study do not demonstrate total recovery at 0.05 mg/L after a 4-week recovery period. Given 2, 4-D's use pattern, additional exposures may occur within this timeframe thus precluding reversibility. Consequently, the agency will continue to use the lowest concentration tested as the LOAEC for this study.

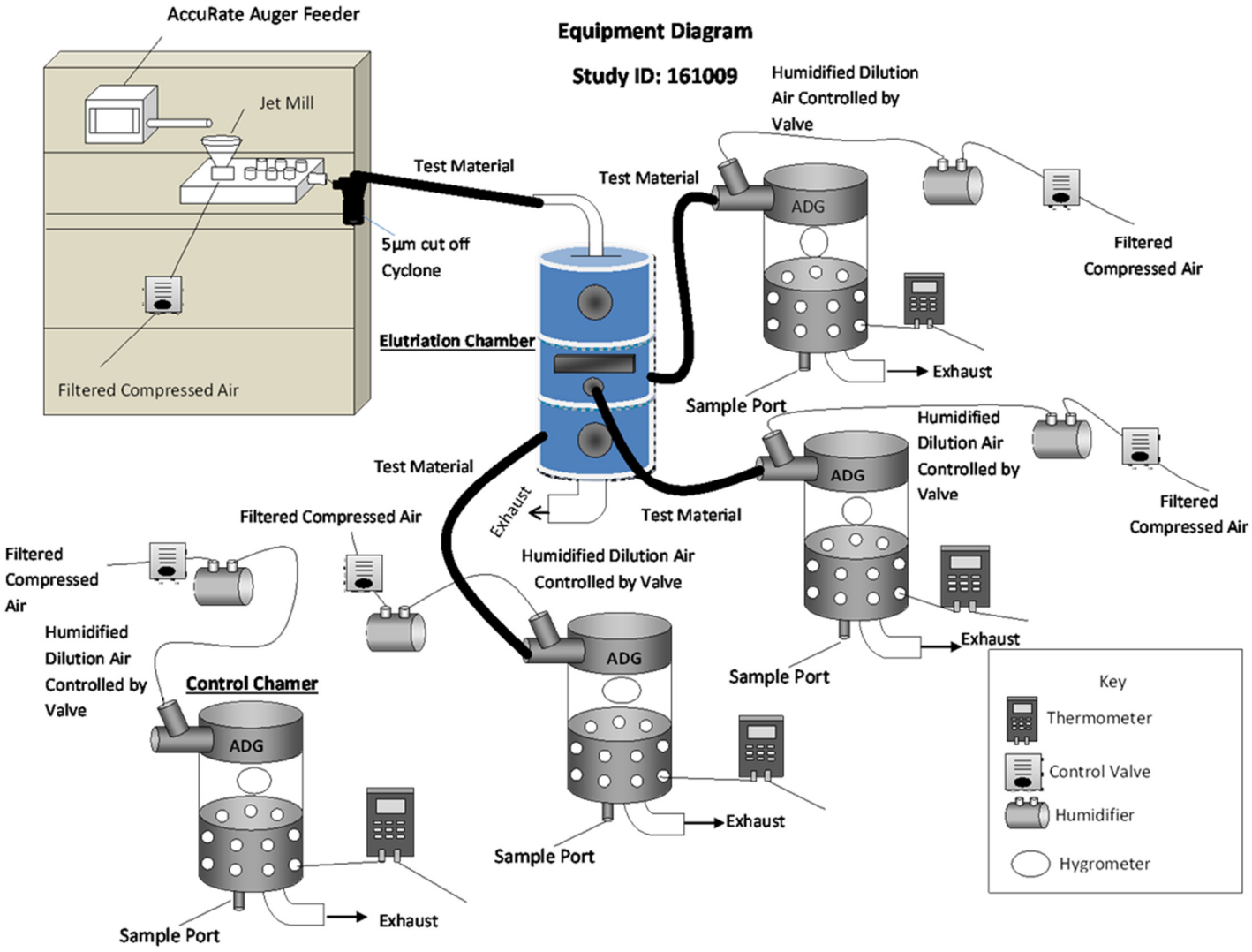
The LOAEC is 0.05 mg/L, based on laryngeal lesions, including persistent incidence of squamous metaplasia of the mucosal epithelium at the base of the epiglottis and inflammation of the laryngeal mucosa at the base of the epiglottis, which were evident following Week 4 of recovery. The NOAEC was not determined.

This study is classified **acceptable / nonguideline** and satisfies the stated purpose of evaluating the time-dependency of recovery from concentration-dependent, point-of-contact epithelial hyperplasia, focal squamous metaplasia and hyperkeratosis, and mixed inflammatory cell influx in the larynx of male Sprague Dawley rats following inhalation exposure to 2, 4-D for 4 weeks.

C. STUDY DEFICIENCIES: No deficiencies were noted based on the stated purpose of this study. However, the registrant's decision to use 0.05 mg/L as the lowest concentration – in spite of the Agency's recommendation to use a lower concentration – failed to identify a NOAEC. As a result, this study only served to confirm the results of the previous subchronic inhalation study.

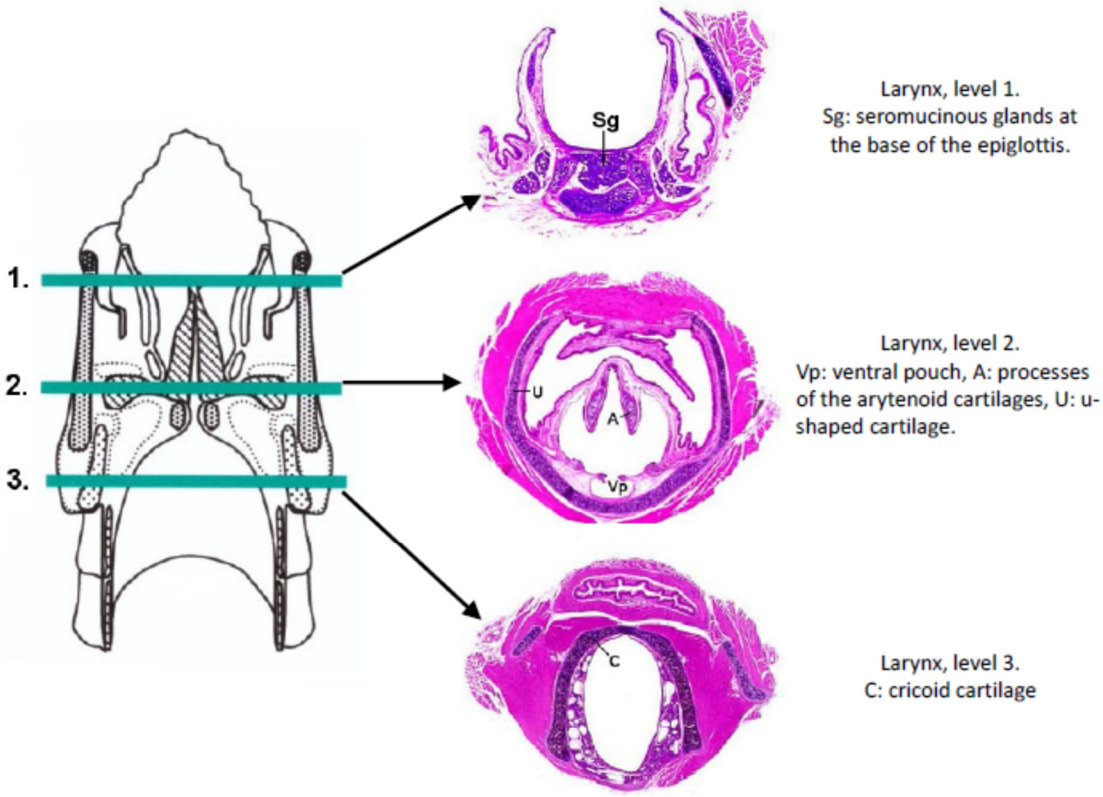
APPENDIX 1

Figure 1. Exposure generation system



APPENDIX 2

Text Figure 1. Anatomic Sites Examined in the Larynx



APPENDIX 2 cont.

Text Table 4. Summary of the Severity Grade Scale to be Used to Assess Treatment-Related Histopathologic Alterations to the Larynx

Severity Grades	Description
Within Normal Limits	The larynx was considered to be within normal limits, under the conditions of the study and considering the age, sex, and strain of the animal concerned.
Very Slight[1]	A very slight grade was used for conditions that were altered from the normal textbook appearance of the larynx, but were of minimal severity and usually with less than 10% involvement of the laryngeal epithelium and/or subepithelial tissues. This type of change would not be expected to significantly affect the overall function of the larynx.
Slight	A slight grade was used for conditions that were of mild severity and usually with less than 25% involvement of the laryngeal epithelium and/or subepithelial tissues. This type of change would not be expected to significantly affect the overall function of the larynx.
Moderate	A moderate grade was used for histopathologic lesions that involve up to 50% of the laryngeal epithelium and/or subepithelial tissues in multiple levels of the larynx, that have the potential for dysfunction of the larynx.
Severe	A severe grade would have been used for histopathologic lesions that involved more than 50% of the laryngeal epithelium and/or subepithelial tissues in multiple levels of the larynx that were deemed to cause clear dysfunction of the larynx.

[1] This severity grade is equivalent to the grade of "minimal" used in the previous study reported by Hoffman (2008).

APPENDIX 3

TABLE 7. Summary of the treatment-related histopathological changes in larynx: Mean Lesion Severity Index ± Std. Error Mean

		End of exposure				1-week recovery				2-week recovery				4-week recovery			
Exposure level (mg/L)		0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3	0.0	0.05	0.1	0.3
Level I	Metaplasia; squamous; epithelium; mucosa	0	2.3 ± 0.2	2.7 ± 0.2	2.7 ±0.2	0	0.67 ± 0.33	1.8 ± 0.3	2.2 ± 0.2	0	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	0	0.50 ± 0.22	0.33 ± 0.21	1.0 ± 0.3
	Hyperkeratosis; epithelium; mucosa	0	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.2	0	0	0.17 ± 0.17	0.50 ± 0.22	0	0	0	0.33 ± 0.21	0	0	0	0
	Inflammation; subacute to chronic; mucosa	0.4 ± 0.2	1.5 ± 0.2	2.0 ± 0.0	2.1 ± 0.1	0.33 ± 0.21	0.83 ± 0.31	1.3 ± 0.2	1.5 ± 0.2	0.33 ± 0.21	0.67 ± 0.33	1.2 ± 0.2	1.7 ± 0.2	0.33 ± 0.21	0.50 ± 0.22	1.0 ± 0.0	1.2 ± 0.3
Level II	Metaplasia; squamous; epithelium; arytenoid	0	0.38 ± 0.26	0.60 ± 0.27	1.2 ± 0.3	0	0	0.33 ± 0.21	0.50 ± 0.34	0	0.17 ± 0.17	0	0.83 ± 0.31	0	0	0	0.17 ± 0.17
	Hyperkeratosis; epithelium; arytenoid	0	0.13 ± 0.13	0.30 ± 0.15	0.60 ± 0.16	0	0	0	0.17 ± 0.17	0	0.17 ± 0.17	0	0	0	0	0	0
	Hyperplasia; squamous; epithelium; arytenoid	0.10 ± 0.10	0	0.30 ± 0.15	1.0 ± 0.3	0	0	0	0	0	0.17 ± 0.17	0	0.17 ± 0.17	0	0	0	0
	Inflammation; subacute to chronic; lamina propria; arytenoid	0	0.38 ± 0.18	0.40 ± 0.16	1.1 ± 0.3	0	0	0	0.33 ± 0.21	0	0.17 ± 0.17	0	0.17 ± 0.17	0	0	0	0.17 ± 0.17

Lesion Severity Index was calculated using the lesion severity score (No Change = 0, Very Slight = 1, Slight = 2, Moderate = 3, Severe = 4) times the number of animals with each score. The mean and standard error of the mean were calculated from those values.
A 0 indicates no animals were observed with the indicated endpoint.